Br J Vener Dis 1983;**59**:145-50

Serological diagnosis of untreated early syphilis

Importance of the differences in THA, TPHA, and VDRL test titres

P J L SEQUEIRA

From the Central Serology Laboratory, Withington Hospital, Manchester

SUMMARY The treponemal haemagglutination (THA) test has been used for 11 years as a routine test for syphilis at a central serology laboratory. Among patients with untreated early syphilis the antibodies detected by the THA test usually appeared later or were of relatively lower titre than those detected by the reagin tests. This finding was confirmed by follow up of patients and by replies to questionnaires and has been incorporated in a computer program. During 1981, using the results obtained on unpaired specimens and minimal other data, 74 of 123 patients who were finally diagnosed as having untreated early syphilis were correctly identified by the computer. Most of those not selected by the computer were patients with early primary syphilis with little or no circulating antibody or with late secondary syphilis with very high titres.

Introduction

At the Central Serology Laboratory (CSL) Manchester, the treponemal haemagglutination (THA) test[1] has been carried out as part of the routine screening procedure for syphilis and positive reactions quantitated since mid-1969. At that time Price's precipitation reaction (PPR) was used as a quantitative test for reagin. It was noticed that most patients with untreated primary and secondary syphilis had higher titres with the PPR than with the THA test. Where the THA titre was higher than the PPR titre the diagnosis was usually treated syphilis or late or latent syphilis. When the Venereal Diseases Research Laboratory (VDRL) test was substituted for the PPR, and allowance was made for the higher sensitivity of the VDRL, this relationship remained.

During the later part of 1980 a computer program was developed using this observation, and further criteria were added on a trial and error basis until the program was highly selective for patients with untreated early syphilis. Each month since January 1981 computer printouts of all new cases of untreated syphilis other than late latent have been sent to the consultants in genitourinary medicine working in the north-west of England. Where available, the diagnosis used is that provided by the clinician. Also included are patients whose diagnosis is not given but who have been selected by the computer program. As these lists should include a patient only once duplicate entries are removed, but some recently treated patients not previously reported are included. Each entry gives the date the specimen was received, the patient's date of birth, the department or area attended, the diagnosis, and the laboratory accession number. The purpose of this study was to examine the success of the diagnostic criteria used and their relationship with the *Treponema pallidum* haemagglutination (TPHA) test.

Address for reprints: Dr P J L Sequeira, Central Serology Laboratory, Withington Hospital, Manchester M20 8LR

Accepted for publication 29 November 1982

Materials and methods

The THA tests, cardiolipin Wassermann reaction (WR), and VDRL tests were carried out on all specimens. The specimens for these three tests were distributed and diluted as a single operation using a hand operated multiple pipetting and diluting machine (Denley Instruments, Billingshurst, West Sussex), which pipettes a row of 10 specimens simultaneously into WHO haemagglutination plates. Using this instrument one operator pipettes 40 specimens in four to five minutes, which allows if necessary the testing of two days' specimens on one day. The volumes distributed were 0·1 ml of undiluted serum for the VDRL, 0·1 ml volumes of a 1/5 dilution of serum for the test and control of the CWR, and 0·3 ml of a 1/30 dilution for the THA test.

VDRL TEST
The test was carried out with Wellcome antigen in WHO plates using double the volumes used in the slide test. Using a dropper 0·033 ml of antigen was added to 0·1 ml of serum in each well and the plates mechanically rotated for five minutes at about 120 rotations per minute. The tests were read with the naked eye on a light box with a tubular 60 watt filament bulb with its centre line 70 mm from the plate bottom. The inside of the box was matt black. The level of illumination was previously set to give the same sensitivity as when the slide test is read with a microscope at × 100, so that a weak positive reaction or quantitative endpoint is seen with the naked eye as a fine precipitate. This method does no detect "rough" negatives.

CARDIOLIPIN WASSERMANN REACTION
The test was carried out according to the Whitechapel technique except for the use of WHO plates. The incubation period was 60 minutes at 37°C before addition of the sensitised cells, the plates were shaken to resuspend the cells after 15 minutes, and the tests read on a numbered white backing sheet after a further 45 minutes.

TREPONEMAL HAEMAGGLUTINATION TEST
The test was carried out as described by Sequeira and Eldridge.[1] The sensitised cells were prepared daily, and 0·2 ml added to the appropriate 0·3 ml dilutions of serum. The test was read after 35-45 minutes' incubation at 37°C. The test was not titrated beyond a titre of 64 from the screen dilution. A description of the micro-THA test is in preparation.

TPHA TEST
The Fujizoki preparation was used according to the maker's instructions. Sensitised cells were added to alternate rows of the quantitative tests so that results were read as fourfold dilutions. The last serum dilution of the quantitative tests was 1/5120 (after addition of the cells).

WELLCOME "SYFHATECT" TEST
This test was carried out according to the maker's instructions. This test is formulated for fourfold dilutions. The last serum dilution of the quantitative tests was 1/1280 (before addition of the cells).

FLUORESCENT TREPONEMAL ANTIBODY-ABSORBED (FTA-ABS) TEST
The FTA-ABS test was carried out using multispot slides with three rows of five "spots." Positive (+ + + +), weakly reactive (+ +), and negative control sera were included on each slide. Tests were read on a Leitz microscope with a 100 watt halogen-quartz light source, oil immersion dark field condenser, × 40 objective, and × 10 eyepieces. The filters used were a KP490 exciter filter and K510 barrier filter.

QUALITY CONTROL
Positive control sera at appropriate dilutions were prepared for each test from locally prepared pools of positive sera standardised against the WHO reference serum kindly supplied by Mme Paris-Hamelin of the Alfred Fournier Institute, Paris. Batches of results where the positive control results deviated one dilution or more from the standard were discarded and the specimens retested.

CLINICAL SPECIMENS
During the course of the study about 103 000 specimens of blood or serum were received. Of these, about 50 000 were collected at routine antenatal examinations and about 35 000 were from patients attending departments of genitourinary medicine. Among the latter, a clinical diagnosis of syphilis was given on 1417 requests, and in a further 955 (as a minimum) the THA and VDRL tests were positive or the FTA test was positive (+ + + + or + + +). These patients were considered to have serological evidence of syphilis. Among requests from other sources a diagnosis of syphilis was given in 293 patients and serological evidence of syphilis was found in a further 830.

For the purpose of the circulars the clinical diagnosis was usually that on the initial request form or the computer diagnosis. The final diagnoses of the patients were obtained from the initial and subsequent requests forms supplemented by the laboratory records. Where no diagnosis was available from these sources a computer generated questionnaire was sent to the clinician concerned. Replies were received for 49 of 53 patients, but the stage of the disease was not given in four.

DATA PROCESSING
The reports and records of the Central Serology Laboratory have been generated on data processing equipment since 1962. Records include a cumulative card file of significant results and coded clinical information. During the study the laboratory's Data General Nova 2 computer was used.[2]

The selection program was run to examine each completed record once. Records were selected if a diagnosis of syphilis other than late latent was recorded and there was no record of treatment or where the following criteria were met: the VDRL titre was two doubling dilutions or more higher than that of the TPH test, the FTA-ABS test was positive (+ + + + or + + +), the CWR was positive, and the patient was under 55 years of age.

Results

As this laboratory uses the THA test and most other laboratories the TPHA test a comparison of these tests and of the new Wellcome Syfhatect was carried out. As the descriptions of these tests use different reporting conventions to indicate titres the results are expressed in units, which are the ratio of the serum dilution at the endpoint and the serum dilution of the screen test. Units therefore indicate the position of the endpoint in the set of dilutions rather than the actual serum dilution. For example, the maker's recommended screen test dilution for the Fujizoki TPHA test is at a final dilution of 1/80. If the endpoint dilution for a specimen was 1/1280 the result has been recorded as 1280/80 = 16 units. This can be calculated at any convenient stage; thus with the Wellcome Syfhatect the screen test contains three volumes of 1/20 dilution to which one volume of sensitised cells are added. If the endpoint is at the third well of the fourfold dilutions the serum dilution is 1/320 and the result would be reported as 320/20 = 16 units. The units for the VDRL would be the reciprocal of the titre as the screen uses undiluted serum.

Table I shows the results of the THA test, TPHA (Fujizoki), Syfhatect, micro-THA (CSL), and VDRL tests on sera from seven patients with untreated primary and seven with untreated secondary syphilis who had been diagnosed by consultants in genito-urinary medicine. These patients were otherwise unselected. The THA, TPHA, and Syfhatect tests were each carried out twice on different working days. The largest discrepancy between a pair of results on one specimen were "doubtful" to 4 units with the TPHA on two specimens.

The largest discrepancy between two tests on one serum was the THA negative and the Syfhatect 4 and 16 units. To compare the sensitivities of the tests the results were scored as 0, 0·5, 1, 2, 3, and 4 for negative, ±, 1 unit, 4 units, 16 units, and 64 units respectively. The average scores in each group of results are given in table I and suggest that the TPHA and Syfhatect tests were of similar sensitivity and the THA rather less sensitive. In only one specimen (204650) were both the THA results significantly weaker than one or both readings of another test. The VDRL test was positive at 1/4 or more with all these specimens.

The results of the THA test and TPHA test using Fujizoki reagents are compared in table II. The 93 sera were from those selected for confirmatory testing over four working days using different batches of THA reagent but one batch of TPHA reagents. The results of the TPHA test are given in conventional final endpoint dilutions and in units. The THA results are in the dilution of the amount of serum in the screen test and in units to allow direct comparison. Thus there was complete agreement with 53 sera shown in the marked diagonal, a difference of one cell of the table with 31 sera, of two cells with eight sera, and of three cells with one serum. When scored as in table I the average scores of the THA and TPHA were 1·42 and 1·55 respectively. In two patients the TPHA test was negative and the THA test positive or weakly reactive, in one the THA test was negative and the TPHA test doubtful. J R Sequeira (personal communication) suggested that if the differences were random the number of agreements and disagreements by one or more cells should fall in a Poisson distribution. The cumulative probabilities (0·58,

TABLE I *Comparison of treponemal haemagglutination results (expressed in units) in patients with primary and secondary syphilis*

Diagnosis	*Laboratory*	*VDRL test*	*THA test**		*TPHA test**		*Syfhatect**		*Micro-THA*
Primary syphilis	210210	4	–	±	±	4	±	±	4
	202452	8	±	1	–	–	1	±	±
	210522	8	–	–	±	4	±	±	±
	205701	16	–	–	±	1	±	1	±
	201768	32	–	–	±	–	–	–	–
	204650	64	–	–	4	4	4	16	4
	202283	128	±	±	1	±	4	1	1
Mean score			0·2		0·7		0·6		0·9
Secondary syphilis	204006	32	–	±	4	4	4	4	1
	208975	64	±	1	4	4	4	4	1
	203740	64	16	64	64	64	64	64	16
	207570	128	64	64	64	64	64	64	64
	299450	256	64	64	64	64	64	64	64
	202526	256	64	64	64	64	64	64	64
	205528	256	64	64	64	64	64	64	64
Mean score			2·9		3·4		3·4		3·0

– = Negative; ± = doubtful; VDRL = Venereal Disease Research Laboratory (test); THA = treponemal haemagglutination (test); TPHA = *T pallidum* haemagglutination assay.
*Tests performed twice on two different working days

TABLE II *Comparison of results of THA and micro-TPHA tests*

TPHA results		*THA test results in serum dilutions (units):*						
Titres	*Units*	*– (–)*	*± (±)*	*1/1 (1)*	*1/4 (4)*	*1/16 (16)*	*1/64 (64)*	*Totals*
–	–	29	1	1	0	0	0	31
±	±	1	1	1	3	0	0	6
1/80	1	0	1	2	3	0	0	6
1/320	4	0	4	8	8	4	0	24
1/1280	16	0	1	0	7	7	2	17
1/5120	64	0	0	0	0	3	6	9
Totals		30	8	12	21	14	8	93

– = Negative; ± = doubtful

TABLE III *Summary of cases included in the laboratory circulars for 1981*

Diagnosis	*Men*	*Women*	*Unspecified*	*Totals*
Untreated primary syphilis	24	3	1	28
Untreated secondary syphilis	24	13	5	42
Untreated early latent syphilis	10	7	1	18
Untreated cardiovascular syphilis	2	3	0	5
Untreated neurosyphilis	7	10	0	17
Untreated early congenital syphilis	0	3	0	3
Untreated late congenital syphilis	4	6	1	11
Early syphilis on laboratory results	50	30	6	86
Other	5	2	0	7
Totals	126	77	14	217

0·90, 0·98, and 0·998) were calculated from this distribution and fit the observed values (53, 31, 8, and 1) with a probability of 0·95 using the χ^2 method.

All the cases included in the circulars for 1981 are summarised in table III. In all, a clinical diagnosis of early syphilis was given on the request form with 88 specimens while a further 86 for whom no clinical diagnosis was given were diagnosed as early syphilis by the computer alone. The seven patients diagnosed as "other" were excluded on initial review of the material, three because of errors in coding, three from the misinterpretation of an unusual code by the program, and one from rediagnosis as untreated late latent syphilis.

The final diagnoses of patients attending departments of genitourinary medicine and the number of each group selected by the computer as untreated early syphilis are shown in table IV. Among the 43 patients diagnosed as untreated primary syphilis, the computer found 25. Among the 18 patients missed, five were seronegative, four had a positive or weakly reactive FTA-ABS test only, and in five the VDRL was positive and the FTA-ABS test weakly reactive or negative. Of the 55 patients with secondary syphilis, the computer found 37. Of the 18 missed, 15 were at a late stage with very high titres in the VDRL and THA tests. Another two of those missed were subsequently found to have been treated, and in one the specimen was too small for the VDRL test to be carried out.

TABLE IV *Final diagnosis in patients attending a genitourinary medicine clinic included in laboratory circulars for 1981*

Final diagnosis	*Total No of patients*	*No with computer diagnosis (untreated early syphilis)*
Untreated primary syphilis	43	25
Untreated secondary syphilis	55	37
Untreated early latent syphilis	25	12
Untreated cardiovascular syphilis	3	0
Untreated neurosyphilis	6	2
Untreated early congenital syphilis	2	0
Untreated late congenital syphilis	4	0
Other stages or treated syphilis	7	5
No or incomplete diagnosis	8	8

Among the 25 patients diagnosed as having early latent syphilis, 13 were missed by the computer, nine because of insufficient difference between the VDRL and THA test titres. Six of these patients formed a group with low titre results significantly different from the remaining patients with untreated early latent syphilis. Therefore of the 123 patients finally diagnosed as having untreated early syphilis, the computer identified 74 (60%).

Of the remaining 2249 specimens from patients who were attending departments of genitourinary medicine and were clinically or serologically diagnosed as syphilis, the computer selected 15 patients (0·7%) as having untreated early syphilis.

Among these 15, we received no diagnosis or no indication of the stage in eight. The remaining seven were two patients with untreated neurosyphilis with evidence of activity in the cerebrospinal fluid and one with untreated late latent syphilis. Three other patients were found to have been treated and one was diagnosed as having treated yaws. Therefore, among the 81 clinically diagnosed patients who were selected by the computer as cases of untreated early syphilis, 74 (91%) were correctly identified.

Among the 50 000 specimens from antenatal patients, serological evidence of syphilis was found in 36 (0·07%), but in only two were the criteria for inclusion in the circular met. The VDRL test was reactive and the THA and FTA-ABS tests negative in 61 (0·1%). These were considered to be false positive reactions as no patient developed other serological or clinical evidence of syphilis. Unfortunately, one patient in the region who received no antenatal care was delivered of an infant with congenital syphilis.

Most of the remaining 18 000 specimens were from sources where routine serological testing for syphilis was not carried out or were "problem sera" from other regions. Among these, 40 (0·2%) met the criteria of the program. As the diagnosis and follow up of these patients is not as consistent as among those attending departments of genitourinary medicine, and the specimens are highly selected, detailed analysis has been limited to patients attending these departments.

Discussion

Passive haemagglutination tests for syphilis have been developed from Rathlev's original method[3 4] using formolised cells of different species, different serum and cell concentrations, and different suspending media. When these different preparations are used as qualitative tests, in accordance with published descriptions or the suppliers' instructions, a remarkable degree of agreement is found both between different tests and different laboratories. This agreement between the various haemagglutination tests at the qualitative level may be because the developers of the tests have adjusted them to detect the minimum significant amount of antibody; however, the various conventions defined in the descriptions for reporting quantitative results has produced the chaos shown in table V.

When a haemagglutination test is developed the serum dilution is only one of many variables in the formulation. The actual concentrations of materials in the wells are technical details only. The important parameter is the position of the endpoint in the set of serial dilutions. If there is agreement in sensitivity between the qualitative tests and the titres are reported in units of the ratio of the endpoint serum dilution and screen test serum dilution, quantitative results become directly comparable (unpublished observations). We therefore recommend that quantitative results of haemagglutination tests for syphilis should be reported in units. These will give the same result if calculated from the serum dilutions before or after addition of the sensitised cells. The last column in table V shows the resulting clarification even with tests of different formulation. If for special purposes a lower serum dilution is used, the results can be represented as a fraction of a unit—for example, a TPHA endpoint at a final dilution of 1/20 would be reported as 20/80 = ¼ unit.

The level of agreement between the haemagglutination tests for syphilis suggest that the criteria for diagnosis of untreated early syphilis used at this laboratory should be applicable at other laboratories, but experience might indicate minor amendments. The criteria become as follows: (*a*) the VDRL test units four or more times the haemagglutination test units; (*b*) the FTA-ABS test positive (+ + + + or + + +); (*c*) the cardiolipin WR positive; (*d*) no record of treatment; (*e*) the patient to be under 55 years of age.

The first two criteria selected 189 specimens from patients attending genitourinary medicine clinics and 106 from other departments; the remaining three criteria excluded 85 and 66 respectively. In all, 23 were excluded on account of age only, 34 because the cardiolipin WR was not positive and 37 because the patients had been treated. A further 94 did not meet

TABLE V *Initial and final serum dilutions in some serological tests for syphilis*

Test	*Screen dilution*		*Dilution at 16 units*		*Conventional report*	*Titre in units*
	Initial	*Final*	*Initial*	*Final*		
THA	1/30	1/50	1/480	1/800	1/16	16
TPHA (Fujizoki)	1/20	1/80	1/320	1/1280	1/1280	16
TPHA (Whitley)	1/20	1/80	1/320	1/1280	1/320	16
Micro-THA	1/20	1/80	1/320	1/1280	1/16	16
Syfhatect	1/20	1/26·7	1/320	1/426·7	1/320	16
VDRL	Undiluted	1/1·03	1/16	1/16·5	1/16	16
Wassermann reaction*	1/5	1/20	1/80	1/320	1/80	16

*Public Health Laboratory Service

two criteria and five did not meet three. The 104 specimens from patients attending genitourinary medicine clinics which met all criteria were from 89 patients.

Only three patients who were reinfected were identified by the laboratory during 1981. Two with clinically diagnosed primary syphilis were selected by the computer program but had titres more typical of secondary syphilis; the third was diagnosed as having secondary syphilis but was not selected by the program as the titres were too high.

While a computer is not required for simple titre comparisons, it made possible the trial of various criteria on large numbers of records and finally the production of the monthly circulars. The selection of patients with secondary syphilis was improved by limiting the THA titres to 64 units; further adjustments resulted in more losses than gains but might be required with other reagents.

Thus, the routine procedures of a laboratory will affect the selection of patients in that the FTA-ABS test is normally carried out for diagnosis only, and therefore follow-up specimens and treated patients will usually be excluded as there would be no FTA-ABS test result. In the event, using these criteria, the laboratory computer selected an average of about 20 patients each month for inclusion in the circular. From these, one to three would be deleted as duplicate entries and about one in 40 subsequently found to be incorrectly diagnosed.

The validity of the method of diagnosis of untreated early syphilis based on the VDRL titre being greater than the haemagglutination titre (in units) is confirmed by the results of the questionnaires which were prepared on the basis of the laboratory results where no diagnosis was given. Of the 53 patients in the questionnaires, data were received on 49. Sixteen had subsequently been diagnosed as having primary syphilis, 14 as having secondary syphilis, and nine as having early latent syphilis. Eight other patients had syphilis, four of unspecified stage, and one had treated yaws. Thus, in 39 of the 45 (87%) replies where the stage of the disease was given the computer diagnosis of early syphilis was confirmed.

About half the patients with untreated early syphilis were selected by the computer because no clinical data were given on the request form. Inspection of the data showed that patients with untreated early syphilis not identified by the computer fell into two groups, those with early primary syphilis with little or no circulating antibody and those with late secondary syphilis with high titre test results. These data are consistent with the antibody pattern selected by the computer representing a phase in the normal development of the disease.

Our findings at the Central Serology Laboratory have shown that it is possible to diagnose 60% of patients with untreated early syphilis by the serological examination of one blood specimen and minimal other information. As the method is based on the VDRL titre being higher than that of the haemagglutination test associated with a positive FTA-ABS test, it follows that the VDRL or equivalent test must be included in the routine serological examination. In only five of the 43 patients diagnosed as having primary syphilis was the FTA-ABS test positive or weakly reactive and the VDRL negative. It would be unwise therefore to base the diagnosis of primary syphilis on the finding of an unconfirmed, unsupported reactive FTA-ABS test result. In most of these patients, however, the diagnosis was supported by positive dark field examination, which remains the method of choice, or by the VDRL also being positive on a confirmatory specimen, or by the clinical findings. Though during the period of this survey the routine use of the FTA-ABS test among contacts and other patients suspected of developing primary syphilis made little difference to the management of the cases, it was still of value as a confirmatory test.

I thank my clinical colleagues for the data used in this report, also the technical and clerical staff of the Central Serology Laboratory for their high standard of work, especially Mrs M Postles for her clerical assistance. I also thank Dr J Cradock-Watson and Dr D R Baird for their comments on this report.

References

1. Sequeira PJL, Eldridge AE. The treponemal haemagglutination test. *Br J Vener Dis* 1973;**49**:242-8.
2. Sequeira PJL. A laboratory data processing system for non-numeric data In: Anderson J, ed. *Medical informatics Europe 1978*. Berlin: Springer-Verlag, 1978.
3. Rathlev T. Haemagglutination test utilizing antigens from pathogenic and apathogenic *Treponema pallidum*. *WHO/VDT/RES/77.65*.
4. Rathlev T. Haemagglutination test utilizing pathogenic *Treponema pallidum* for the serodiagnosis of syphilis. *Br J Vener Dis* 1967;**43**:181-5.